1. PURPOSE: To set for the procedure for the amplification of DNA using the AmpFlSTR® Yfiler™ PCR Amplification Kit (Yfiler).

2. SCOPE: The Yfiler system will be used by the NCSBI Forensic Biology Section on Forensic Evidence to obtain profiles from male individuals.

3. SAFETY

   3.1. AmpliTaq Gold DNA Polymerase may cause eye and skin irritation. Exposure may cause discomfort if swallowed or inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

   3.2. During thermal cycler operation, the temperature of the heated cover can be as high as 108 °C, and the temperature of the sample block can be as high as 100 °C. Keep hands away from the heated cover and sample block.

   3.3. Hi-Di Formamide: Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

4. DEFINITIONS

   Y-STRs: Short Tandem Repeat sequences from the male-specific Y-chromosome.

5. REFERENCE DOCUMENTS

Applied Biosystems. AmpFℓSTR® Yfiler™ PCR Amplification Kit User's Manual. 2006.

Applied Biosystems. ABI Prism® 3100 Analyzer and 3100-Avant Genetic Analyzer User Reference Guide.

Applied Biosystems. ABI Prism® Genetic 3100 and 3100-Avant Genetic Analyzers – Protocols for Processing AmpFℓSTR® PCR Amplification Kit PCR Products. User Bulletin. 2003.

Applied Biosystems. Quantifiler™ Kits: Quantifiler™ Human DNA Quantification Kit and Quantifiler™ Y Human Male DNA Quantification Kit User's Manual. 2003.

Butler, J.M. Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers. 2nd ed. Burlington, MA: Elsevier Academic Press, 2005.

Julio J. Mulero Ph.D, Chien Wei Chang Ph.D, Lisa M. Calandro M.P.H, Robert L. Green B.A, Yixin Li Ph.D, Cassie L. Johnson M.S, Lori K. Hennessy Ph.D (2006) Development and Validation of the AmpFℓSTR® Yfiler™ PCR Amplification Kit: A Male Specific, Single Amplification 17 Y-STR Multiplex System. Journal of Forensic Sciences 51 (1) , 64–75 doi:10.1111/j.1556-4029.2005.00016.x

NCSBI Validation Studies for Yfiler and Quantifiler Y.

6. PROCEDURE

6.1 Master Mix Preparation

6.1.1 Thaw the PCR reaction mix and primer set, then vortex 3 to 5 seconds and centrifuge briefly before opening the tubes.

6.1.2 Vortex the AmpliTaq Gold DNA Polymerase for 3 to 5 seconds and centrifuge briefly.

6.1.3 At medium speed, briefly vortex the PCR reaction mix, primer set, and AmpliTaq Gold® DNA polymerase reagents supplied in the Applied Biosystems AmpFℓSTR® Yfiler™ PCR Amplification Kit. Centrifuge briefly before opening the tubes.

6.1.4 Calculate the volume of each component needed to prepare the reactions, using the table below.

Component	Volume Per Reaction (µL)
AmpFlSTR Yfiler Kit PCR Reaction Mix	(Number of samples + 2) x 9.2
AmpFlSTR Yfiler Kit Primer Set	(Number of samples + 2) x 5.0
AmpliTaq Gold® DNA Polymerase	(Number of samples + 2) x 0.8

Note: Additional reactions included in the calculations to provide excess volume for the loss that occurs during reagent transfers.

6.1.5 Pipette the required volumes of components into an appropriately sized polypropylene tube.

6.1.6 Vortex the PCR master mix for 3 to 5 seconds, then centrifuge briefly.

6.2 PCR Sample Set Up

6.2.1 Dispense 15 µL of the PCR master mix into each reaction well. Use MicroAmp® Reaction Tubes or a MicroAmp® Optical 96-Well Reaction Plate or equivalent.

Note: Known Female Standards should not be amplified and analyzed.

6.2.2 Add 10 µL of sample/TE to the appropriate wells to achieve a final DNA concentration of 0.5-1.0 ng.

6.2.3 Add 10 µL 007 (supplied male positive control @ 0.1ng/µL) to the appropriate well.

6.2.4 Add 1 µL of 9947A (supplied negative control) to the appropriate well.

6.2.5 The final reaction volume should be 25 µL in each well.

6.2.6 Centrifuge the plate at approximately 2000 rpm for about 20 seconds in a tabletop centrifuge. Amplify the DNA.

6.3 PCR Amplification

6.3.1 Program the thermal cycling conditions.

Initial Incubation Step	Cycle (30 Cycles)			Final Extension	Final Hold
	Denature	Anneal	Extend		
Hold	Cycle			Hold	Hold
95 °C 11 min	94 °C 1 min	61 °C 1 min	72 °C 1 min	60 °C 80 min	4 °C

6.3.2 Load the plate into the thermal cycler.

6.3.3 Choose the appropriate cycling program for Yfiler.

6.3.4 Close the heated cover.

6.3.5 Start the run.

6.4 Capillary Electrophoresis

6.4.1 Calculate the volume of Hi-Di™ Formamide and GeneScan®-500 LIZ® Internal Size Standard needed to prepare the samples, using the table below.

Reagent	Volume (µl) per Run of 16 Samples
GeneScan-500 LIZ Size Standard	~ 7
Hi-Di™ Formamide	176

6.4.2 Pipette the required volumes of components into an appropriately sized polypropylene tube.

6.4.3 Vortex the tube, then centrifuge briefly.

6.4.4 Into each well of a MicroAmp® Optical 96-Well reaction plate, add:

- 9 µL of the formamide: size standard mixture
- 1 µL of PCR product or Yfiler Allelic Ladder

Note: For blank wells, add 10 µL of Hi-Di™ formamide.

6.4.5 Seal the reaction plate with appropriate septa and then briefly centrifuge the plate to ensure that the contents of each well are mixed and collected at the bottom.

6.4.6 Heat the reaction plate in a thermal cycler for 3 min at 95 °C.

6.4.7 Immediately place the plate on ice for 3 min. or hold at 4°C in the thermal cycler.

6.4.8 The same run modules are used with autosomal STRs.

6.4.9 YSTRs and STRs may be run together on the same plate.

6.4.10 If an increased or decreased injection time is used, the injection time for the associated reagent blanks must be adjusted accordingly.

6.5 Analyzing Sample Files with GeneMapper ID **Open File**

6.5.1 Open GeneMapper ID v3.2.1 or current version.

6.5.2 Click on File> Add samples to project.

6.5.3 Go to My Network Places

6.5.4 Entire Network> Microsoft Windows Network> NCBCI0094 and choose the 3100/3130 on which the run was performed.

6.5.5 Go to “Appliedbio”>3100>Data Extractor.

6.5.6 Find your run by date and the run number.

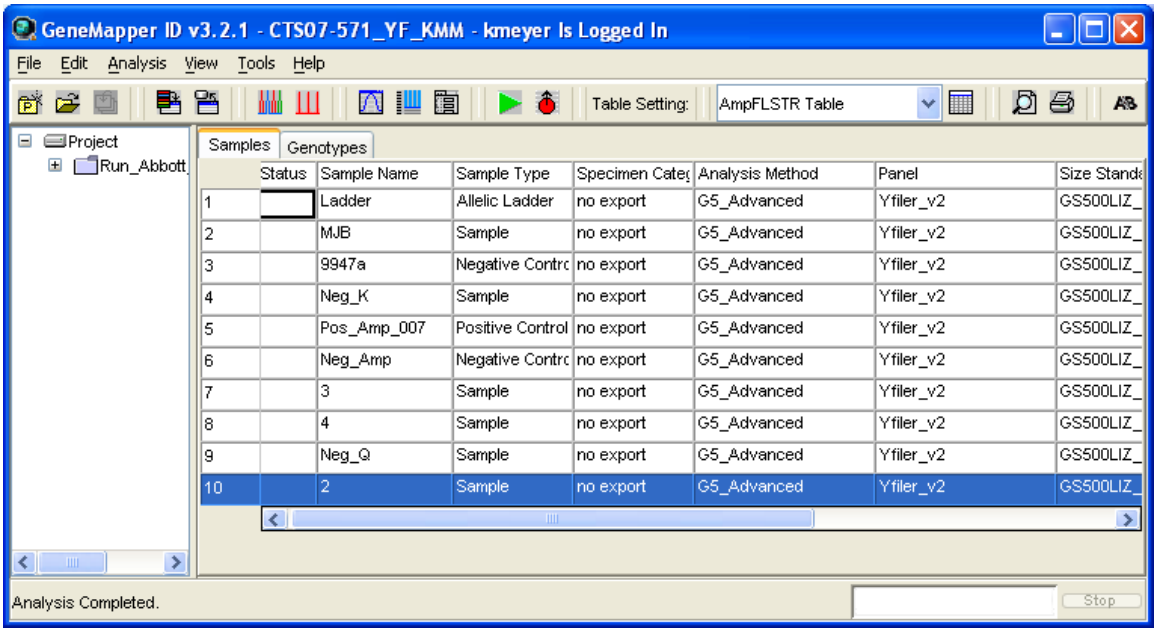
6.5.7 Highlight Run Folder or Specific Sample from the run. Add samples to list and click “Add”.

6.5.8 Apply analysis setting to the samples in the project

Parameter	Analysis Method
Table Setting	Select **AmpFlSTR Table** from the drop-down list.
Sample Type	Select the sample type.
Analysis Method	**G5_Advanced**
Panel	**Yfiler_v2**
Size Standard	**G5_HID_GS500**

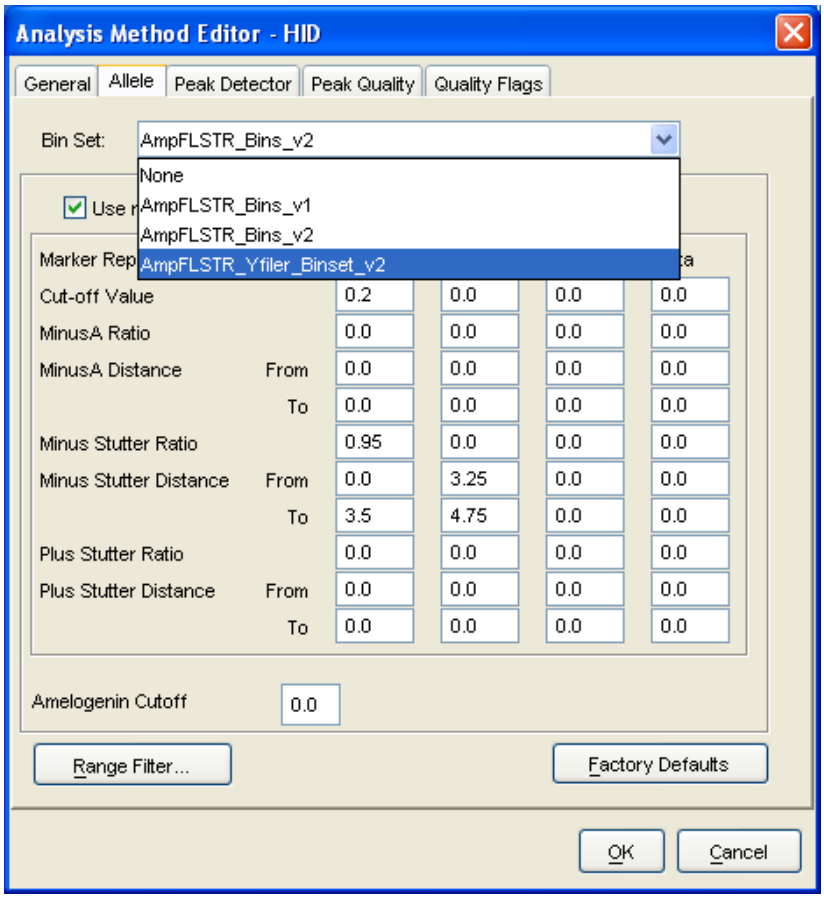
Labeling samples: Check to make sure:

- “Sample Type” and the “Sample Name” match.
- Ladder is labeled as “Allelic Ladder”.
- 007 is labeled as “Positive control”.
- Negative Female Amp control (9947A) is labeled as “Negative control”.
- Negative Amp control is labeled as “Negative control”.
- Samples are labeled as “Samples”.



6.5.9 To Analyze a project:

Once all samples are labeled properly,



Click (Analyze), type a name for the project (in the Save Project dialog), then click **OK** to initiate analysis.

- The status bar displays progress of analysis:
  - As a completion bar extending to the right with the percentage indicated
  - With text messages on the left
- The table displays the row of the sample currently being analyzed in green (or red if analysis failed for the sample). See Figure 5-1.
- The genotypes tab becomes available after analysis.

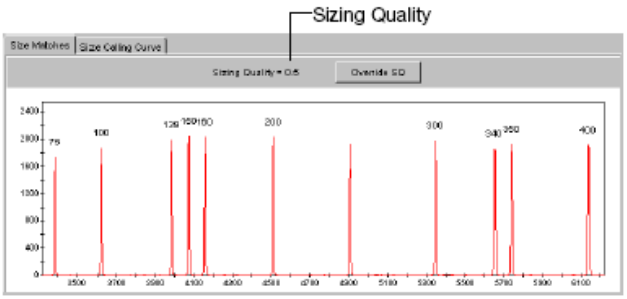
6.5.10 Examination of Samples

6.5.10.1 Select Header Sample name and go to View>Raw Data.

6.5.10.2 Review each samples raw data visually.

6.5.10.3 Duplicated samples (two positive amp. controls or two ladders were run) and samples that are overblown (and are to be re-analyzed later) may be removed from the project. If a sample is deleted from the project the reason for the deletion must be detailed in the analyst's notes. To delete a sample from the project, go to Project node, select the name of the sample to be deleted, chose Edit>Delete from project.

6.5.11 Examine Size Standards

1.	Select **Edit** > **Select All** and click (Size Match Editor) to view the size standard for all samples.
2.	Notice that the Sizing Quality value equals 0.5. 
3.	View the peak assignments for each sample: a. Press the down arrow key to scroll through the samples on the left side of the Size Match Editor screen. b. Confirm that the size for the peaks in the **HID_Classic_GS500(-250)** size standard (GeneScan™-500 LIZ® Size Standard) are correctly assigned for each sample.

6.5.12 Examine the 250-bp peak

6.5.12.1 Select> All Samples [Ctrl A], click Display Plots icon (Multi-colored icon).

6.5.12.2 Select the Overlay Liz plot from the plot settings drop-down list. Click the Hide All icon, and then click only the orange square to show only the orange peaks. This is the LIZ size standard.

6.5.12.3 Drag down the upper box to allow you to view both sets of peaks at the same time.

6.5.12.4 The upper box contains the size standard peaks for the controls in your project and the lower box contains the size standards for the samples.

6.5.12.5 Click underneath the 250-bp peak in the upper box. Compare these results then compare the results in the lower box.

6.5.12.6 The selected peak will be highlighted in the table below the boxes.

6.5.12.7 You can scroll through the samples with the up and down arrow keys.

NOTE: The 250 bp peak was not defined in the size standard. The purpose of checking the 250-bp peak is to determine if the samples and controls are all within +/- 0.5 bp from the ladder. The 250-bp peaks should consistently overlap. In a typical run, the 250-bp peaks all fall within a size window of +/- 0.5 bp. If the samples are too far off (the 250 bp size standard is >+/- 0.5 bp) the sample should be re-injected.

6.5.12.8 Go back to the project in the Project window.

6.5.13 Examining Data

6.5.13.1 To check the Precision Quality Value of the samples click on the “Genotypes” tab and scroll to the right and check the Precision Quality Value (PQV).

6.5.13.2 Anything with a yellow ▲ or a red stop sign needs to be reviewed carefully.

6.5.14 Examine the Ladder (Note: Ensure the Plot setting is set on “HID Genotype”

6.5.14.1 Highlight the ladder and click the Display Plots icon  (Mulit-colored icon).

6.5.14.2 Scroll through the ladder double checking it corresponds with the key
(Separate handout).

6.5.14.3 If a section of the Ladder comes up with a yellow ▲, make sure the alleles are called correctly and if so you can right click on the yellow ▲ and click "yes" to override the results, and change the yellow ▲ to a green ■.

6.5.14.4 Continue until all sections of the ladder are green ■s.

6.5.15 Examine the Controls

Examine controls. If the controls did not work properly, then they must be re-analyzed (re-injected, re-amped, or re-extracted).

6.5.16 Examination of Samples

6.5.16.1 If the ladder and the controls all worked properly you can continue by analyzing the samples as you did the ladder and the controls.

6.5.16.2 If there is a problem, look at the date by viewing the Raw Data [Ctrl F2] or view all the samples at once by viewing the samples under **HID Genotyping** (from the pull down menu)

6.5.16.3 If removing a called allele due to OL (Off Ladder) or pull up, stutter, dye blob, spike, etc, changing or adding an allele. Left click to choose it, then right click to delete, change, or rename.

6.5.16.4 You must note in the comment box that appears the reason the allele call is changed.

6.5.16.5 The Raw Data showing the primer peaks and Size Standards of samples that are negative (have no observable peaks, including negative controls) should be printed and included in the analysts notes.

6.5.17 Save Project

Once all samples have been reviewed save the project. To save click "File"> "Save".

6.5.18 Export Tables

Note: Two tables must be created to export into Excel

6.5.18.1 Click on the "**Samples Tab"** and from the pull down menu choose ***"SBI Casework Table"***

6.5.18.2 File> Export Table

6.5.18.3 Place the table in the correct folder

6.5.18.4 For the second table, click on the "**Genotypes Tab"** and from the pull down menu choose ***"Case Allele Table"***. **Note:** This table may be sorted prior to

exporting into Excel. This may be accomplished by choosing “Edit” then “Sort by Name”.

6.5.19 **Convert tables to Excel Format**

6.5.19.1 Open MS Excel; then using the open command from the File menu, open the exported table. When you do this, you will be prompted to convert the file to an Excel format using the “Text Import Wizard”.

6.5.19.2 There are 3 steps to this wizard:

6.5.19.2.1 Make sure “Delimited” is checked, click “Next”.

6.5.19.2.2 Make sure “Tab” is checked, click “Next”.

6.5.19.2.3 Click on “Text” in the Column Data Format section, then click “Finish”.

6.5.19.3 Use the “Save as” command in the File menu to save your data as an Excel worksheet.

6.5.20 **To Create the Allele Call Table**

6.5.20.1 Open the GeneMapper Results Worksheet for Y-STRs.

6.5.20.2 Click on the Results Tab at the bottom left corner of the document.

6.5.20.3 Click on your Exported table document and select all (Ctrl + A).

6.5.20.4 Copy all the data (Ctrl + C).

6.5.20.5 Go back to the GeneMapper Results worksheet, click on cell A1 in the “Results” section, and paste your results (Ctrl + V).

6.5.20.6 Click on the “Allele Call Table” tab at the bottom to see your results displayed in a table format.

6.5.20.6.1 Use the “Save as” command in the File menu to save the document, rename it, and put it into your run folder.

6.5.20.7

6.5.20.8 From the Project window, select **File > Add Samples** to Project to navigate to the disk or directory containing the sample files.

6.5.20.9 Apply analysis settings to the samples in the project.

6.5.20.10 Click (Analyze), type a name for the project (in the Save Project dialog), then click **OK** to initiate analysis.

6.5.20.11 Click (Analyze), type a name for the project (in the Save Project dialog), then click **OK** to initiate analysis.

7. APPENDICES

None

Revision History		
Effective Date	Revision Number	Reason
Revision History		
April 10, 2008	00	Original Document

APPROVAL SIGNATURES	Date
Author/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	
Name/Title (Print)	
(Signature)	